

# Heavy metals in sediment, water and the European glass eel, *Anguilla anguilla* (Osteichthyes: Anguillidae), from Loukkos River estuary (Morocco, eastern Atlantic)\*

by

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**ABSTRACT.** - Comparisons of metal concentrations (Fe, Zn, Cu, Cr, Pb and Cd) were made in sediment and water with those in tissues of glass eel (*Anguilla anguilla* Linnaeus, 1758) collected in five stations from Loukkos estuary (Morocco, eastern Atlantic). Concentrations of selected metals were measured using an atomic absorption spectrophotometer (AAS). Significant correlations ( $p < 0.05$ ) between Cu-Pb were observed in sediment, Fe-Pb, Cr-Pb and Cr-Fe in water and Zn-Fe in fish. The metal levels found in tissues in the glass eel were below the legal limits for human consumption, but can induce severe dysfunctioning for the eel.

**RÉSUMÉ.** - Métaux lourds dans les sédiments, l'eau et les civelles d'*Anguilla anguilla* (Osteichthyes : Anguillidae) de l'estuaire de la rivière Loukkos (Maroc, Atlantique est).

Les concentrations de six éléments métalliques (Fe, Zn, Cu, Cr, Pb et Cd) ont été comparées dans le sédiment et l'eau avec celles des tissus de civelles (*Anguilla anguilla* Linnaeus, 1758) prélevés au niveau de 5 stations de l'estuaire du bas Loukkos (Maroc). Les différents éléments métalliques ont été analysés par spectrométrie d'absorption atomique (SAA). Une corrélation significative ( $p < 0,05$ ) a été trouvée entre Cu-Pb dans les sédiments, Fe-Pb, Cr-Pb et Cr-Fe dans l'eau et Zn-Fe dans les civelles. Les teneurs de métaux trouvés dans les tissus de civelles étaient en dessous des limites légales pour la consommation humaine, mais peuvent induire de sérieux dysfonctionnements chez l'anguille.

Key words. - Anguillidae - *Anguilla anguilla* - Morocco - Loukkos estuary - Heavy metals - Sediment - Water.

Heavy metals are considered as the most important pollutants in the aquatic environment. They can concentrate in fish species from water, food and sediment (Harderson and Written, 1998; Fernandes *et al.*, 2007), especially in eels, which depend on freshwater and estuarine areas during juvenile growth phase. Consequently, anthropogenic impact such as pollution by heavy metals and other chemical components could influence the decline of species: to date commercial catches of European eel represent less than 1% compared to the 1970s (Maes, 2005). The impact of pollutants in eels is not well known (Robinet and Feunteun, 2002), may be due to the fact the species accumulates various heavy metals (Zimmermann *et al.*, 2004) and consequently appears to be useful as a metal accumulation indicator (Gunkel, 1994). The biological cycle of European eel is complex, with four life stages and two metamorphoses, which have been well

described by Tesch (2003). Reproduction takes place in the Sargasso Sea from where larvae drift back towards the European and North African coasts following oceanic currents, such as the Gulf Stream. After the metamorphosis of the larvae into glass eels. Post larval stage that corresponds to the freshwater colonizing stage (Pierron *et al.*, 2007). The eels reach the juvenile growth stage (yellow eel) in continental habitats (Élie, 1979). This stage can last from several years to more than 20 years depending on the hydrosystem.

So, the estuarine ecosystem plays a fundamental role in the life cycle of the European eel, particularly on glass eel recruitment. Unfortunately, because of their geographical position, estuaries represent a receptacle of pollution from the upstream basin slopes. The Gironde estuary (south-western France), for example, has been subjected for more than a century to polymetallic contamination and contains particu-

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larly high levels of cadmium (Audry *et al.*, 2004; Baudrimont *et al.*, 2005).

In Morocco no study was carried out to determine the accumulation of metals in European eel. The Loukkos River, Moroccan Atlantic coast, is currently considered as the most polluted of the Moroccan (Merja Zerga) shore (Yahyaoui *et al.*, 2006). The purpose of this study is to analyse and compare metal concentrations in water and sediment with those in tissues of glass eels collected from five stations in the Loukkos estuary.

## MATERIALS AND METHODS

### Study site

The catchment basin of the Loukkos River ( $35^{\circ}9' - 25^{\circ}14'N$  and  $6^{\circ}5' - 6^{\circ}3'W$ ) covers an area of 3750 km<sup>2</sup> (Fig. 1). The estuary is 20 km long, with a fluvial harbour located on the left side, 1 km from the mouth. This harbour is bordered southeastern by an industrial zone and southwestern by Larache city. The adjacent region, Loukkos plain, supports extensive farming activity extending for almost 20,000 ha (Cheggour *et al.*, 2005). The annual average Loukkos River output, recorded in the upper sector, is 60 m<sup>3</sup>/s, ranging from 0.5 m<sup>3</sup>/s (August-September) to 200 m<sup>3</sup>/s (January-February). The annual solid flow ranges between 0.2 and 6.10<sup>6</sup> tons (Snoussi, 1984). During the dry period, inputs to the estuary come only from the bordering marshes.

According to the classification of Pritchard (1955), the Loukkos estuary may be described as "an estuary with a salt corner" in the wet season and as "a partially mixed estuary" in the dry season (Snoussi, 1984).

Five stations in the estuary were investigated (Fig. 1), related to the presence of industry (congealment, cannery, and fish flour), active fishing sites, garbage of factories, agriculture and domestic worn-out waters.

### Sediment

Core samples up to 20 cm in length were collected from 15 sampling sites in March, May and July 2006 (three sampling stations in each of the five stations selected) using polyvinyl chloride corers (Cabrera *et al.*, 1992). The corers were immediately sealed and stored at 4°C until arriving at the laboratory.

In the laboratory, the cores were extruded and sectioned. The first 5 cm section of each core was used in this study (Meyerson *et al.*, 1981). Sections were air-dried (Thomas *et al.*, 1994) and sieved with a 63- $\mu$ m nylon mesh, and the fraction < 63  $\mu$ m was chosen for chemical analysis (Rauret *et al.*, 1988; Thomas *et al.*, 1994). The samples underwent acid digestion (HNO<sub>3</sub>-HClO<sub>4</sub>) in an automatic microwave digestion system.

Trace metals (e.g., copper, chromium, lead and cadmium) in digested sediment samples were determined by a Graphite Furnace Atomic Absorption Spectrophotometer (AAS) (AWG 120) and iron and zinc contents were analysed

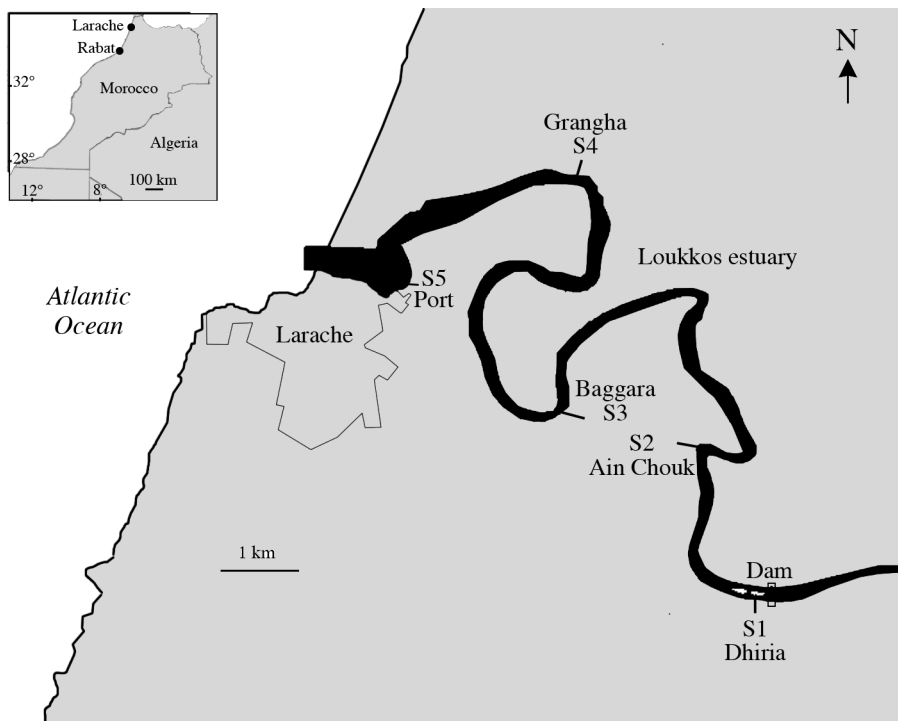


Figure 1. - Site of sample collection (Loukkos River estuary in Morocco).

Table I. - Mean concentrations (mg/kg dry mass) of heavy metals in sediment. Fe concentration in mg/g.

Metal	S1	S2	S3	S4	S5
Zn	135.4 ± 34.5	136.8 ± 25.1	106.1 ± 10.5	98.2 ± 6.4	109.4 ± 3.7
Pb	101.4 ± 2.8	88.0 ± 11.2	104.4 ± 21.7	116.3 ± 8.2	124.1 ± 0.2
Fe	31.6 ± 11.5	28.8 ± 10.1	27.3 ± 13.2	26.7 ± 15.7	36.7 ± 5.3
Cr	91.1 ± 3.4	72.7 ± 8.0	47.5 ± 64.2	106.1 ± 7.8	58.3 ± 77.4
Cu	27.9 ± 2.6	20.1 ± 6.4	27.3 ± 1.6	23.8 ± 2.1	27.1 ± 0.7
Cd	1.8 ± 0.6	1.4 ± 0.7	1.9 ± 1.3	1.9 ± 0.8	2.2 ± 1.2

Table II. - Correlation matrix among monitored metals in sediment (Pearson (n)). The values in bold are significantly different from 0 (level of significance alpha = 0.05).

Variables	Zn	Pb	Fe	Cr	Cu	Cd
Zn	<b>1</b>					
Pb	-0.439	<b>1</b>				
Fe	0.190	0.354	<b>1</b>			
Cr	0.018	-0.097	-0.175	<b>1</b>		
Cu	-0.152	<b>0.670</b>	0.307	-0.299	<b>1</b>	
Cd	-0.287	0.058	-0.184	0.271	0.139	<b>1</b>

by flame (VARIAN AA 240 Z). Blanks were included in each batch of analysis. Calibration standards were regularly performed to evaluate the accuracy of the analytical method.

### Water

Water samples were collected from the same sampling sites as the sediment and fishes, using 1l acid-leached polyethylene bottles. The samples collected in March, May and July 2006 were filtered through a 0.45 µm membrane filter, acidified with 2 ml of concentrated HNO<sub>3</sub> and stored at room temperature until analysis. The samples were analyzed by graphite furnace AAS, with Zeeman background corrector, after preliminary metal concentration according to the method described by Sturgeon *et al.* (1980).

Copper, lead and cadmium was analysed by Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) (Perkin Elmer 4100ZL) using Zeeman-effect background correction. Iron and zinc were analysed by Flame-AAS. Chromium was analysed directly, without preconcentration, by GFAAS (Perkin Elmer 4100ZL) using Zeeman-effect background correction and operation conditions recommended by the manufacturer. Sample concentrations of chromium were, however, below the detection limit (0.42 µg/l) and are not presented here.

### Glass eels

In this study glass eels (*A. anguilla*) averaging 7.5 ± 0.2 cm in length and 0.165 ± 0.006 g in weight (mean ± S.E., n = 225) were used. Samples of 15 fish for each station were collected in March, May and July 2006 from the Loukkos estuary. Soon after capture, specimens were dried at 80°C and crushed to uniform particle size before analysis (Miao *et al.*, 2001). For analysis, two grams of flesh of *A. anguilla* were dissolved in a solution of nitric

acid P.A. (HNO<sub>3</sub>: H<sub>2</sub>O = 2:1) at 130°C for 2 h. Undissolved particles were filtered off and the solution diluted to 25 ml.

Cadmium, chromium, copper, and lead were measured by graphite furnace (AWG 120) AAS, while iron and zinc were measured using flame (AAS VARIAN AA 240 Z).

### Reagents and quality assurance

All reagents used were of analytical grade (Merck). Standard working solutions of the different elements analysed were prepared from the corresponding 1000 mg/l Merck Titrisol solution.

The digestion and analytical procedures were checked by analysis of standard reference materials (sediment: CRM-277, Community Bureau of Reference; fish: DORM-2, National Research Council; and seawater: CASS-3, National Research Council Canada). Replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 88% and 104% for fish, 92% and 98% for sediment and between 95% and 102% for water. In the present study, both a Person Correlation was conducted using SPSS for windows release 12.0.

## RESULTS

### Sediment

The highest concentration levels of most of the metals (iron, zinc, lead, chromium, copper and cadmium) analysed (Tab. I) were found in the sediment from the station 1, due to fact that this station was polluted by toxic industrial wastes. Higher mean iron concentrations (36.70 mg/g dry mass) were obtained in the station 5, and lower mean cadmium concentrations (1.40 mg/kg dry mass) were detected in station 2. The sediment metal concentration followed the order:

Table III. - Mean concentrations ( $\mu\text{g/l}$ ) of heavy metals in water from five sampling stations. Mean  $\pm$  standard deviation.

Metal	S1	S2	S3	S4	S5
Zn	0.03 $\pm$ 0.03	0.03 $\pm$ 0.02	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02	0.03 $\pm$ 0.02
Pb	0.18 $\pm$ 0.03	0.19 $\pm$ 0.04	0.25 $\pm$ 0.16	0.15 $\pm$ 0.03	0.13 $\pm$ 0.02
Fe	0.05 $\pm$ 0.02	0.04 $\pm$ 0.02	0.04 $\pm$ 0.03	0.02 $\pm$ 0.01	0.01 $\pm$ 0.004
Cr	6.66 $\pm$ 0.57	7.00 $\pm$ 1.73	7.00 $\pm$ 2.00	4.33 $\pm$ 1.52	5.66 $\pm$ 2.08
Cd	0.12 $\pm$ 0.05	0.08 $\pm$ 0.01	0.12 $\pm$ 0.10	0.33 $\pm$ 0.48	0.12 $\pm$ 0.08
Cu	2.80 $\pm$ 0.20	0.70 $\pm$ 0.60	2.50 $\pm$ 0.80	4.00 $\pm$ 1.50	3.50 $\pm$ 1.30

Table IV. - Correlation matrix among monitored metals in water (Pearson (n)). The values in bold are significantly different from 0 (level of significance  $\alpha = 0.05$ ).

Variables	Zn	Pb	Fe	Cr	Cd	Cu
Zn	<b>1</b>					
Pb	0.143	<b>1</b>				
Fe	-0.209	<b>0.564</b>	<b>1</b>			
Cr	0.156	<b>0.560</b>	<b>0.552</b>	<b>1</b>		
Cd	0.207	0.189	0.092	0.155	<b>1</b>	
Cu	-0.031	-0.369	-0.390	-0.404	-0.073	<b>1</b>

Table V. - Metal concentrations (mg/kg dry mass) in *Anguilla anguilla* from five sampling stations. Mean, standard deviation and value limits.

	Fe	Zn	Cu	Cr	Pb	Cd
S1	86.95 $\pm$ 33.23 (63.60-111.00)	40.40 $\pm$ 0.99 (39.70-41.10)	1.45 $\pm$ 0.21 (1.30-1.60)	2.57 $\pm$ 0.02 (2.56-2.59)	0.03 $\pm$ 0.01 (0.02-0.03)	0.38 $\pm$ 0.05 (0.35-0.42)
S2	54.25 $\pm$ 0.21 (54.10-54.40)	36.60 $\pm$ 1.84 (35.30-37.90)	1.95 $\pm$ 1.34 (1.00-1.90)	3.01 $\pm$ 0.76 (2.48-3.55)	0.02 $\pm$ 0.001 (0.03-0.03)	0.43 $\pm$ 0.03 (0.41-0.45)
S3	179.75 $\pm$ 59.61 (138.00-222.00)	47.75 $\pm$ 7.28 (42.60-52.90)	1.05 $\pm$ 0.35 (0.80-1.30)	2.78 $\pm$ 0.54 (2.40-3.17)	0.03 $\pm$ 0.01 (0.02-0.30)	0.44 $\pm$ 0.02 (0.43-0.46)
S4	67.90 $\pm$ 2.69 (66.00-69.80)	37.90 $\pm$ 6.36 (33.40-42.40)	4.20 $\pm$ 3.68 (1.60-6.80)	2.90 $\pm$ 0.41 (2.61-3.19)	0.02 $\pm$ 0.01 (0.02-0.03)	0.47 $\pm$ 0.11 (0.37-0.52)
S5	80.35 $\pm$ 23.97 (63.40-97.30)	39.60 $\pm$ 0.71 (39.10-40.10)	0.45 $\pm$ 0.42 (0.16-0.75)	2.66 $\pm$ 0.30 (2.45-2.87)	0.03 $\pm$ 0.01 (0.03-0.04)	0.45 $\pm$ 0.03 (0.43-0.48)

Table VI. - Correlation matrix (Pearson: n) among monitored metals in *Anguilla anguilla*. The values in bold are significantly different from 0 to a level of significance  $\alpha = 0.05$ .

Variables	Fe	Zn	Cu	Cr	Pb	Cd
Fe	<b>1</b>					
Zn	<b>0.976</b>	<b>1</b>				
Cu	-0.138	0.040	<b>1</b>			
Cr	0.502	0.430	0.187	<b>1</b>		
Pb	0.081	-0.013	-0.874	-0.585	<b>1</b>	
Cd	-0.517	-0.345	0.411	-0.818	0.040	<b>1</b>

(S1) > (S4) > (S5) > (S2) > (S3). The general ranking of metal accumulation in sediment was: Fe > Zn > Pb > Cr > Cu > Cd. Significant positive correlation was obtained between Cu-Pb ( $p \leq 0.05$ ; Tab. II)

### Water

Metals concentrations in water are given in table III. Higher concentrations of lead in water were detected in station 3 (0.25  $\mu\text{g/l}$ ). Bearing in mind the absence of industrial dumping of lead in the area, this may be due to a heavy-traffic

road across the station. Concentration of chromium, copper, lead, cadmium and iron were significantly higher in the water from the station 3 than in station 2. Higher mean chromium concentrations (7.00  $\mu\text{g/l}$ ) were obtained in station 3. However, lower mean zinc concentrations (0.02  $\mu\text{g/l}$ ) were noted in station 3. The water metal concentration followed the order: (S3) > (S1) > (S4) > (S5) > (S2). The general order of monitored metal accumulation was: Cr > Cu > Pb > Cd > Fe > Zn. Significant positive correlation was found between Fe-Pb, Cr-Pb and Cr-Fe ( $p \leq 0.05$ ; Tab. IV).

### Glass eels

Mean metal concentrations in specimens from each station are given in table V. Specimens collected in the station 3 had considerably higher mean metal content (iron, copper, chromium, lead and cadmium) than fish from the station 2, with the exception of zinc. The station 3 showed the highest mean concentrations of iron and zinc and also exhibited the highest concentration in water. Higher mean iron concentrations (179.75 mg/kg dry mass) were obtained in station 3, and lower mean lead concentrations (0.02 mg/kg dry mass) were detected in station 2. The glass eel metal concentration followed the order: (S3) > (S1) > (S5) > (S4) > (S2). The general order of monitored metal bioaccumulation was: Fe > Zn > Cr > Cu > Cd > Pb. This order might be attributed to the different uptake, metabolism and detoxication processes in the fish. Significant positive correlation was obtained between Zn-Fe ( $p \leq 0.05$ ; Tab. VI).

## DISCUSSION

### Sediment

High concentrations of zinc, chromium and copper were found in the sediment from the station 1 and were comparable only to those obtained in other areas affected by pyrite pollution, such as the Huelva estuary (Pérez *et al.*, 1991), or because of significant industrial dumping as in Gdansk Bay in Poland (Glasby and Szefer, 1998). By contrast, in stations 2 and 3, the levels of these metals were low, similar to those found in sediment from other non-polluted areas (Forstner and Wittman, 1983). An exception to this was lead, which reached considerably lower concentrations ( $88 \pm 11.2$  mg/kg dry mass) in the station 2 than in station 3 ( $104.4 \pm 2.17$  mg/kg dry mass). This is because the station 3 is near a heavily travelled highway and probably as has been documented in another study (El Morhit *et al.*, 2008). The increase of the salinity in this station contributed to the release of lead from sediment to water.

Among the metals analysed in Loukkos river estuary, the levels of cadmium in sediment are the lowest in the five stations. Data on cadmium in Moroccan aquatic systems are less abundant than for other metals. The few recent studies that were carried out in estuaries (Tahiri *et al.*, 2005) pointed out the role of human activities in cadmium loadings, in agreement with other studies in Pennsylvania (USA) (Bopp and Biggs, 1981). The amounts of iron are high in all the studied stations, consistent with previous studies in Morocco (Cheggour *et al.*, 1990; Texier *et al.*, 1994; Cheggour *et al.*, 2005). Iron is the most abundant metal in Loukkos estuary because it is one of the most common elements in the earth's crust and the sediment from the station 5 has a high pyrite (FeS<sub>2</sub>) content (Snoussi, 1984). According to Cabrera *et al.* (1999), pyrite oxidation produces sulphate and the Fe<sup>2+</sup> ion,

which is oxidised to Fe<sup>3+</sup> by microorganisms such as *Thiobacillus ferrooxidans*. High concentrations of chromium were found in the sediment from the station 4 and were comparable only to those obtained in other areas affected by industrial wastewater pollution, such as the Om Rbiâ estuary (Jadal *et al.*, 2002), Port Jackson estuary (Hatje *et al.*, 2003) and Bouregreg estuary (Tahiri *et al.*, 2005).

### Water

In the water, the metal contents measured in stations 1, 2 and 3 were considerably higher than those previously reported in marine water (Nicolai *et al.*, 1999; Cotté-Krief *et al.*, 2000). By contrast, the station 1, is affected by contamination from the waters of draining of the rice fields (El Blidi *et al.*, 2006), showed zinc, lead, iron and cadmium concentrations that agree with those reported in other studies for coastal and estuary waters. For example, the mean lead levels of 0.18 µg/l in stations in our study are lower to those reported by Usero *et al.* (2003) in salt marshes on the southern Atlantic coast of Spain. Works done by Morales *et al.* (1999) and by Usero *et al.* (2003) had shown cadmium levels similar to those in our study. Copper concentrations (0.7 µg/l in station 2 and 2.5 µg/l in station 3) were of the same order of magnitude as those found by Nicolai *et al.* (1999) in the Rhone River estuary in France, Usero *et al.* (2003) in salt marshes on the southern Atlantic coast of Spain. The range of chromium levels was 4.3 µg/l (in station 4) to 7 µg/l (in station 3). These values are higher than those recorded by Morales *et al.* (1999) in the Gulf of Valencia (0.16 µg/l). However, chromium, levels higher than ours were found in the Po River estuary in the Adriatic Sea (Italy) by Pettine *et al.* (1997).

### Glass eels

Since eels are long lived, they can accumulate high concentrations of pollutants, so that less polluted waters can also be potentially harmful. The higher metal concentrations were found in glass eels from the Loukkos estuary. The glass eels from station 3 was found to have a high relative metal concentration (iron, zinc, copper, lead and cadmium) compared to that from the other stations. There are runoff inputs at station 3. It is difficult to assess the significance of the higher concentration of zinc in station 3 compared to station 1 glass eels since the concentration of this metal are influenced by factors other than their environmental availability. For example fish exposed to heavy metals (such as cadmium) synthesize metal-binding proteins (metallothioneins) in the liver, which not only bind cadmium, but also zinc (Noel-Lambot *et al.*, 1978). Thus, increased liver concentrations of zinc and copper may be related to elevated metallothioneins synthesis, and not necessarily to elevated environmental concentrations. In addition, zinc and copper concentrations appear to be independent of levels of these metals in natural (or exper-

imental) environments because of regulation mechanisms versus non-essential metals, such as lead and cadmium, concentrations of which depends in the organism mainly on their environmental levels. The physiological concentrations of zinc and iron are under regulatory control and eels have been considered unsuitable as bioindicator organisms for these metals (Bruslé, 1990). Iron was found to have the highest concentration of all the monitored metals in glass eels. Iron concentrations similar to those of the Odiel reservoirs were found in the livers of grey mullets from the Huelva estuary (AIQB, 2001), an area polluted by industrial and mining effluents. Nowadays, the limit for iron and zinc in the standard of the EC (2006) are not yet defined.

Among the Loukkos River estuary, the station 4 showed the highest mean concentrations of copper (4.20 mg/g dry mass) in glass eels. It is to be noted that this station presented the highest levels in water (2.70 mg/g dry mass) and sediment (23.80 mg/g dry mass). Metayer *et al.* (1984) found copper concentrations of (0.55-104.90 mg/kg dry mass) in the elvers and yellow eels of S. Gilla lagoon (Sardinia). These are higher than the concentrations of (1.82 mg/kg dry mass) in eels from Loukkos estuary, which suggests they are probably within the normal physiological range, and hence not of toxicological significance to the eels. But in another study, Sanchez *et al.* (1998), reported a copper concentration of 34 mg/kg dry mass in eel livers and 185 mg/kg dry mass in those of brown trout, an area polluted by industrial mining effluents. In our study, the level for copper in EC is not defined.

Chromium levels were relatively high in tissues as compared to the concentration found in the water. However, the chromium concentration in the sediment was much higher. This could be suggestive of a large quantity of chromium uptake via the food chain because of the bottom feeding habits of *A. anguilla*. Chromium is used in industry for electroplating, steelmaking alloys, in chrome plating, rubber manufacturing, leather tanning and for fertilisers (Babich *et al.*, 1982). The toxicity of chromium is dependant on its chemical speciation and thus associated health effects are influenced by the chemical form of exposures (Holdway, 1988). The level for chromium in EC is not defined.

The European Community proposed threshold values of metal concentrations in fish muscle only for nonessential metals (e.g., lead and cadmium). The threshold values are expressed as mg/kg wet weight and are 0.05 for cadmium and 0.2 for lead (EC Directive 02/221/EEC). For an easier comparison, these values have been converted into mg/kg dry mass by multiplying them by 5 (a value was obtained from several analyses).

The mean value of lead bioaccumulation in glass eel tissues varied from 0.026 mg/kg in station 2 to 0.031 mg/kg dry mass in station 5. In another study from the Adour estuary, higher values of lead were noted in glass eels (Bureau *et*

*al.*, 2007). The mean concentrations of lead reported from five rivers in the UK eels ranged between 2.4 and 4.1 mg/kg dry mass, which are higher than the median values described here. Although the latter concentrations are below the 1.5 mg/kg (dry mass) threshold suggested by Mason and Barak (1990), it would be prudent to investigate further the transfer and uptake of this toxic metal, particularly since the station 3 site has an additional input of lead shot from hunting (Pain, 1991) as well as receiving lead *via* riverine and atmospheric sources (Martin *et al.*, 1989; Guieu *et al.*, 1993), in addition to inputs from rivers such as the Loukkos River. The effect of salinity and the mode of application (oral *versus* aqueous) on the lead accumulation in different eel tissues was investigated by Zimmermann *et al.* (1999). Waterborne as well as dietary lead exposure causes an increase in the metal levels of different eel tissues. The mode of lead uptake had a significant influence on the distribution of lead in the fish tissues. This distribution may be due to the different microhabitats (Zimmermann *et al.*, 1999). Lower values of lead (0.026-0.031 mg/kg dry mass) were recorded in our study when compared with previous studies. In a polder (Tjeukemeer), liver concentrations of lead (170 mg/kg dry mass) of eels, higher to those of keeping the same site, were deemed unfit for human consumption (Badsha and Goldspink, 1988). In the Loire estuary, the concentrations of lead (2.5 µg/g dry mass) were followed along the food chains, which lead to the eel (Amiard *et al.*, 1982). It should be noted that the mean content lead evaluated with 0.026 mg/kg dry mass in *A. anguilla* did not exceeded the standard of the EC fixed at 0.2 mg/kg of the wet weight.

Cadmium concentrations of more than 0.05 µg/g (wet weight) are the maximum acceptable level set by the EC directive on cadmium levels in fish muscle. The overall cadmium levels in the muscle tissues of all the specimens analysed were well over the proposed limit values. Although the concentrations of cadmium in the muscle tissue of the station 4 and station 5 glass eels were not examined in this study, levels in muscle have been reported to be higher than those of eels from 11 out of 12 rivers examined in England (Barak and Mason, 1990). Surprisingly, few studies have focused on the impact of pollution on eel. Moreover, these later were restricted to the impact of pesticides (Gimeno *et al.*, 1995; Ceron *et al.*, 1996; Fernandez-Vega *et al.*, 1999; Pierron *et al.*, 2007) whereas eels are also recognized to be highly contaminated by heavy metals, especially by cadmium, in several European estuaries compared with other species (Barak and Mason, 1990; Usero *et al.*, 2003; Durrieu *et al.*, 2005). Factors affecting the bioavailability of cadmium are very complex, but an understanding of them is crucial if appropriate strategies for the management of contaminated wetlands must be developed. The factors which can influence the bioavailability of cadmium to aquatic species include salinity (Lawrence and Hemingway, 2003) and

eutrophic states (Pierron *et al.*, 2008). The eels have the ability to concentrate metals in their tissues when they live in areas heavily contaminated. So, in our study, the highest concentration of cadmium (0.47 mg/kg dry mass) was recorded in the station 4, which focuses industrial agglomerations, confirming previous studies. Near an industrial complex near Bristol Severn (UK), eel livers showed highly charged cadmium (25–123 mg/kg dry mass) according Romeril and Davis (1976). Pierron *et al.* (2008) have shown cadmium bioaccumulation in gills at low metal water concentration (2 µg/l). At the gene level, cadmium exposure mimics the effect of hypoxia since we observed a decrease in expression of genes involved in the respiratory chain and in the defence against oxidative stress (Pierron *et al.*, 2007).

Experimental poisoning showed that the eels were much more sensitive to cadmium (lethal concentrations of 445–NOEL µmol/dm<sup>3</sup> according to Noel-Lambot and Bouqueneau, 1977). However, the toxicity of some metals appeared variable depending on the salinity, being higher in fresh water and low in salt water (Denuit *et al.*, 1981). In contrast, for glass eels, we only found significant difference for cadmium in station 5 and cadmium in station 1 concentrations, with the highest concentrations measured in glass eels from station 5 (0.45 mg/kg dry mass) and the lowest in station 1 (0.38 mg/kg dry mass).

#### **Metals correlation between sediment, water and glass eels**

Metal concentrations in tissues of glass eel were lower than those in surrounding sediments. Overall, metals which are absorbed to superficial sediments that are ingested by glass eel may be assimilated, with efficiencies dependent on the sediment type, the animal species and its physiology, and the metal itself. Metals are not subjected to assimilation and hence are not bioavailable to benthic organisms (Griscom *et al.*, 2002).

The assessment of trace metals and their correlations in Loukkos estuary reflected the degree of pollution, which is considered by many regulatory agencies to be one of the largest risks to the aquatic environment. The mean levels of iron, zinc, copper, chromium, lead and cadmium in the Loukkos estuary were lower than the guidelines for drinking-water quality (WHO, 2006), which the mean (Fe, Zn, Cu, Cr, Pb and Cd) concentrations in the water consumption are higher (300, 3000, 1000, 50, 10 and 3 µg/l, respectively).

Based on guidelines, direct use of water from the Loukkos estuary without treatment may aggravate poor health of sensitive groups (Savory and Wills, 1991). For example, the criterion for lead in water for domestic use is 0 to 1.70 g/l (FEPA, 1991). At levels > 100 g/l, possible neurological damage in fetuses and young children may occur (Fatoki *et al.*, 2002). For some of the metals studied in the surface sediments, the metal levels in our study were higher than the

metal levels in the sediments of Montevideo Harbour, Uruguay (Muniz, *et al.*, 2004), Taylor Creek, southern Nigeria (Okafor and Opuene, 2006), Bouregreg estuary (Tahiri *et al.*, 2005) and Om Rbia estuary (Jadal *et al.*, 2002). Higher concentrations of lead (88–124.1 mg/kg dry mass) and cadmium (1.4–2.2 mg/kg dry mass) were found in the sediment from Loukkos estuary, compared with those recorded by RNO (1995) in France (60 mg/kg and 0.15 mg/kg dry mass, respectively). Furthermore, the study sites are located in the discharge points of Loukkos estuary reflecting the cumulative effects of dispersed inputs from Loukkos River. In spite of the levels of trace metals in the sediments, we can deduce that the sediments presented concentrations that were at the Severe Effect Level (Persaud *et al.*, 1992) and may cause adverse biological effects except for iron, zinc and copper respectively.

For the levels of cadmium, lead and chromium, inter-metal correlations appear to be different in the environmental segments. While, inter-metal relationship exists between Cu-Pb ( $r = 0.670$ ) for the sediments ( $p \leq 0.05$ ; Tab. II); Fe-Pb ( $r = 0.564$ ), Cr-Pb ( $r = 0.560$ ) and Cr-Fe ( $r = 0.552$ ) for the water ( $p \leq 0.05$ ; Tab. IV). Glass eels ( $p \leq 0.05$ ; Tab. VI) show a Zn-Fe significant correlations ( $r = 0.976$ ), which is higher to correlation coefficients reported elsewhere (Hung *et al.*, 2001; Liu *et al.*, 2003; Okafor and Opuene, 2006). This implies that uptake for these metals may be a direct mechanism in glass eels. The above observations might suggest that the absorption mechanism of trace metals at Loukkos estuary sediments are mainly controlled by chemical adsorption, rather than physical or deposition of metals with sediments. Metal ions can associate with the ligands via the functional groups such as  $-\text{OH}$ ,  $-\text{NH}_2$ ,  $-\text{CO}_2\text{H}$  of the organic matter in the sediment and generate stable organic-metals (Riffaldi *et al.*, 1983).

From the foregoing, we can conclude that metal concentrations in the glass eel can be useful as bioindicators of the degree of pollution in marine ecosystems, which is in line with the results of other research works (Metayer *et al.*, 1984; Amiard-Triquet *et al.*, 1988; Barak and Mason, 1990; Bruslé, 1990; Mason and Barak, 1990; Bonhommeau *et al.*, 2008).

#### **CONCLUSIONS**

Analytical results showed that the heavy metals contents of the glass eels are below the maximum limits set by European legislation for fish. However, the limits for cadmium remain higher in the glass eels and in sediments. This could be an essential cause for the disappearance of the organism from aquatic systems. The presence of trace elements at low levels in the aquatic ecosystem could influence the sensibility of the glass eels since these elements are often toxic even

at low concentrations. Nowadays, glass eel is endangered in almost countries of the world especially by European countries. This situation should spur Moroccan authorities to prohibit their capture and researchers to investigate other possible causes of the decline of this organism in aquatic systems such negative effects of climatic changes and biotope conditions and the possible implication of the organic contamination with chemical substances especially pesticides residues. Indeed, an investigation on the latter subject is under study by our research group to assess completely the situation in Loukkos River estuary.

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